REMARKS

Claim 1 has been cancelled. Claims 30-52 are newly added.

Support for new claims 30-52 is found in original claims 1-17, 19-22. and 30-31.

The amendment of the paragraph beginning on page 1, line 2 was made to state the relationship of the present application to serial no.09/127,926.

Secondly, Table numbers 1-5 were duplicated in Tables appearing later on in the application, i.e., on pages 87-92. Applicants have corrected this typographical error, by renumbering these Tables sequentially. Thus, the Table number of the Table appearing in the paragraph beginning at page 87, line9, has been changed from Table 1 to Table 11. Similarly, the Table numbers appearing on page 88, line1, page 89, line 1, page 90, line 1 and page 91, line 1 have been changed as follows: Table 2 has been changed to Table 12 (page 88); Table 3 has been changed to Table 13 (page 89); Table 4 has been changed to Table 14 (page 90) and Table 5 has been changed to Table 15. In addition, references in the text to Table 1 and Table 2 on pages 91-92 have been changed to Tables11 and 12 to be internally consistent.

Applicants submit the amendments to the specification do not raise new issues requiring further examination. The Tables mislabeled with numbers 1-5 were present in the original application. The change in numbers is required to properly reference the Tables cited in the application.

Attached hereto is a marked-up version of the changes made to the specification and the claims by the preliminary amendment. The attached page is captioned **"Version with markings to show changes made."**

The Commissioner is authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1300 (Our Order No. A-65353-6/RFT/RMS/RMK).

Please direct any calls in connection with this application to the undersigned at (415) 781-1989.

Dated: 3/19/01

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

Paragraph beginning at line 2 of page 1 has been amended as follows:

This application claims the benefit [is a continuing application] of U.S.S.N.s 60/043,464, filed April 11, 1997, 60/054,678, filed August 4, 1997, 60/061,097, filed October 3, 1997, [09/058,459, filed April 10, 1998, and] 60/087,561, filed June 1, 1998, and is a continuing application of U.S.S.N. 09/127,926, filed July 31, 1998 and 09/058,459, filed April 10, 1998.

Paragraph beginning at page 87, line 9, with the following rewritten paragraph:

Table [1]11. *DEE determined optimal sequences for the core positions of Gβ1 as a function of* $\Delta h_{0.9}{}^{a}$

Paragraph beginning at page 88, line 1, with the following rewritten paragraph:

Table [2]12. *DEE determined optimal sequences for the core positions of Gβ1 as a function of* $\Delta h_{1.0}{}^{a}$

Paragraph beginning at page 89, line 1, with the following rewritten paragraph:

Table [3]13. *DEE determined optimal sequences for the core positions of Gβ1 as a function of* $\Delta\Omega_{0.9}{}^{a}$

Paragraph beginning at page 90, line 1, with the following rewritten paragraph:

Table [4]14. *DEE determined optimal sequences for the core positions of Gβ1 as a function of* $\Delta\theta_{0.9}{}^{a}$--

Paragraph beginning at page 91, line 1, with the following rewritten paragraph:

Table [5]15. *DEE determined optimal sequences for the core positions of Gβ1 as a function of* $\Delta\sigma_{0.9}{}^{a}$

Paragraph beginning at page 91, line 21, with the following rewritten paragraph:

The optimal sequence for the ten core positions of Gβ1 that is calculated using the native backbone (i.e., no perturbation) contains three conservative mutations relative to the wild-type sequence (Table [1]11). Y3F and V39I are likely the result of the hydrophobic surface area burial term in the scoring function. L7I reflects a bias in the rotamer library used for these calculations. The crystal structure of Gβ1 has the leucine at position 7 with a nearly eclipsed χ_2 of 111°. This strained χ_2 is unlikely to be an artifact of the structure determination since it is present in two crystal forms and a solution structure (Gronenborn et al., 1991; Gallagher et al., 1994). Our rotamer library does not contain eclipsed rotamers and no staggered leucine rotamers pack well at this position. Instead, the side-chain selection algorithm chose an isoleucine rotamer that conserves the χ_1 dihedral and is able to pack well. We expect the removal of the strained leucine rotamer to stabilize the protein, a prediction that is tested in the experimental section of this work. The sequences that result from varying individual super-secondary structure parameter values show two notable trends. Small variations in the parameter values tend to have little or no effect on the calculated sequences. For example, varying $\Delta h_{0.9}$ from -0.25 to -1.00 Å (Table [1]11)

and $\Delta h_{1.0}$ from +0.25 to +1.25 Å (Table 2) has no effect on the calculated sequences which demonstrates the side-chain selection algorithm's tolerance to small variations in the initial backbone geometry. Large variations in the parameter values tend to result in greater sequence diversity. For example, $\Delta h_{1.0}$[+1.50Å] contains six out of ten possible mutations relative to Gβ1 (Table [2]12). The apparently anomalous result that occurs for $\Delta h_{0.9}$ at -1.25 and -1.50 Å, an increase in core volume, is explained by the observation that translating the helix towards the sheet plane results in creating a pocket of space in the vicinity of position 20 that ultimately leads to the observed A20V mutation.

Please replace the paragraph beginning at page 92, line 10, with the following rewritten paragraph:

Experimental validation of the designed cores focused on seven of the Δh-series mutants which contain between three and six sequence changes relative to Gβ1. The designed sequences resulting from $\Delta\Omega$, $\Delta\theta$ and $\Delta\sigma$ perturbations are, however, in many cases identical to various Δh-series sequences. Typical far UV circular dichroism (CD) spectra are shown in Figure 15. $\Delta h_{0.9}$[-1.00Å], $\Delta h_{0.9}$[0.00Å], $\Delta h_{0.9}$[+0.75Å] and $\Delta h_{0.9}$[+1.00Å] have CD spectra that are indistinguishable from that of Gβ1 while $\Delta h_{0.9}$[+1.50Å], $\Delta h_{1.0}$[+1.50Å] and $\Delta h_{0.9}$[-1.50Å] have CD spectra similar to that of Gβ1 suggesting that all of the mutants have a secondary structure content similar to the wild-type protein. Thermal melts monitored by CD are shown in Figure 16. All of the mutants have cooperative transitions with melting temperatures (T_m's) ranging from 53 °C for $\Delta h_{0.9}$[+1.50Å] to 91 °C for $\Delta h_{0.9}$[0.00Å] (Table [1]11). The T_m for Gβ1 is 85°C. The measured T_m's for $\Delta h_{0.9}$[-1.50Å] and $\Delta h_{0.9}$[+1.50Å] are for 56 residue proteins compared to 57 residue proteins in all other cases (see Methods and materials) which results in T_m's that are estimated to be about 2 °C higher than what would be expected for the corresponding 57 residue proteins based on the T_m difference between the 56 and 57 residue versions of Gβ1. The removal of the strained leucine at position seven (L7I) along with the increased hydrophobic burial generated by the Y3F and V39I mutations in $\Delta h_{0.9}$[0.00Å] result in a protein that is measurable more stable than wild-type Gβ1. The extent of chemical shift dispersion in the 1D ^{1}H NMR spectrum of each mutant was assessed to gauge each protein's degree of native-like character (Fig. 5). All of the mutants, except $\Delta h_{0.9}$[+1.50Å], have NMR spectra with chemical shift dispersion similar to that of Gβ1 suggesting that the proteins form well-ordered structures. $\Delta h_{0.9}$[+1.50Å] has a spectrum with broad peaks and no dispersion, which is indicative of a collapsed but disordered and fluctuating structure or non-specific association. All seven mutant proteins retain their ability to bind IgG as measured by binding to an IgG-Sepharose affinity column. The stability and native-like character of $\Delta h_{0.9}$[-1.50Å] and $\Delta h_{1.0}$[+1.50Å] indicate that the sequence selection algorithm is sufficiently robust to tolerate Δh perturbations that are as large as 15% of Gβ1's native height super-secondary structure parameter value of 10 Å.

In the Claims:

Claim 1 has been canceled.